RESEARCH ARTICLE

SAMRIDDHI Volume 13, Special Issue 2, 2021 Print ISSN : 2229-7111 Online ISSN : 2454-5767

Screening of Heartwood of Tectona Grandis Linn for Antifungal Activity

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ABSTRACT

Medicinal plants have always been a subject of interest for the human being from ancient times due to health benefits. Researchers found that people in different regions of the world used the same or similar plants for health benefits. Medicinal plant research includes many fields like drug discovery, ethnobotany, traditional and indigenous medicines. During the corona pandemic, we realised that there are no effective therapies available for new and remerging infectious diseases. Public health is badly affected due to drug- resistant microorganisms and the appearance of new disease- causing microbes. Due to the increasing side effects of synthetic drugs and uncontrolled infectious diseases, researchers have taken interest in natural sources of medicines and have screened a large number of plants for new bioactive compounds. Natural products have been a well-known source of various molecules in the process of drug discovery and many bioactive natural product derivatives. Stereochemical and structural properties of natural products are characteristics that are responsible for exploring novel molecular diversity. One of the successful strategies for the investigation of new medicinal agents from plants includes the screening of plant extract, fractions followed by the purification of the constituents. Several plants and their products have been evaluated for their antimicrobial and antioxidant activity. In the present study, the ethanolic extract of the heartwood of Tectona grandis Linn. was screened for antifungal activity

Keywords: Agar-well diffusion method, Antifungal activity, Ketoconazole, Tectona grandis Linn.,

SAMRIDDHI : A Journal of Physical Sciences, Engineering and Technology, (2021); DOI : 10.18090/samriddhi.v13spli02.2

INTRODUCTION

Scientist, researchers, pharmacists, and academicians have a global challenge for the search of new and more effective natural antibiotic agents since many infectious agents becoming resistant to synthetic drugs and also create side effects. Ayurvedic medicines [1]-[8] are used to treat several diseases [9] because the herbs are safe for human consumption as mentioned in Ayurvedic texts. People are familiar with their local herbs and medicinal plants to cure illness, as they have ancient references and scientific evidence about their safety and clinical efficacy [10]. Indian local flora also has a wide potential for being used against corona for prophylaxis or as adjuvant [11].

Plants play a very important role in modern medicinal sciences [12], in the introduction of new therapeutic agents [13], [14], and anti-HIV agents[15]. *Tectona grandis Linn.* belongs to the family Verbenaceae, commonly known as “sagwan” or “teak”. Teak is considered as a major constituent in many folklore medicines. *Tectona grandis Linn.* is native to India, Myanmar, and South-East Asian

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How to cite this article : Singh, R., Verma, K.K., Choudhary, A. (2021). Screening of Heartwood of Tectona Grandis Linn for Antifungal Activity.

SAMRIDDHI : A Journal of Physical Sciences, Engineering and Technology, Volume 13, Special Issue (2), 111-114.

Source of support : Nil

Conflict of interest : None

countries and probably the most widely cultivated high-value heartwood (HVH) in the world [16],[17]. It is one of the most important heartwoods of the world and it is a big tree which distributed in all tropical and subtropical regions. This plant is medicinally important and many reports claim to cure several diseases according to Indian traditional systems of medicines. Its wood is acrid, laxative, sedative and beneficial in piles, leucoderma, and dysentery. It have anthelmintic, analgesic, anti-diabetic, anti-inflammatory and expectorant properties [18]-[21]. The powder of teak wood [22] alleviates skin inflation caused by Melanorrhaea usitatissema [23]. It is also applied to swollen eyelids. The bark is useful in scabies while its flowers are useful in scabies bronchitis, urinary discharges [24] and promotes hair growth [25]-[27]. Roots are useful in the retension of urine and anuria [28],[29]. The oily product of wood chips is used for the treatment of eczema and ringworm. The bark of *Tectona grandis Linn.* is useful in scabies. Flowers are useful in urinary discharges and bronchitis. Seeds and flowers are diuretics. The seed extract is also used as a lotion for eye troubles. The powder of teak wood [30] is beneficial in skin inflammation. It also shows toxicity against termites [31] increased by modifying the responsible compound anthraquinone nucleus [32]. The World's major medical threats are infectious diseases and the situation has further been complicated with time as we feel during the corona pandemic. Fungi are found to occur everywhere in the environment which is unavoidable and causes infection in plants as well as animals. There are ~20 fungi that cause >99% of human fungal infections, although ~ 600 different fungi have been reported to cause infection. Amongst these fungal pathogens, species of *Candida* and *Aspergillus* are the most common causing invasive life-threatening infections. Hence, all screening assays include these fungi. In spite of a large number of antifungal drugs in the market, there remains a need for drugs that are more effective and exhibit broad-spectrum efficacy.

This study was therefore designed to investigate the antifungal activity of the heartwood extracts of *Tectona grandis Linn.* against some infectious fungi like *Aspergillus flavus, A. niger, Candida albicans, Penicillium chrysogenum, Trichophyton rubrum* with ketoconazole as the reference marker.

EXPERIMENTAL

Preparation of Test Extracts

For antifungal screening, powdered heartwood of Tectona grandis Linn. were extracted with ethanol. The ethanolic extract was concentrated in vacuo, fractionated with pet. ether, benzene, ethyl acetate and the residue was re-extracted (2 x 8 hr) for complete exhaustion. Further, the extracts/fractions were pooled individually and dried in vacuo.

All the extracts were stored at 4°C in a refrigerator until screened for a particular activity. However, their final concentration was prepared in the respective solvents, before use.

Source of Test Organisms

Pure cultures of test fungi namely *Aspergillus flavus, Aspergillus niger, Candida albicans, Penicillium chrysogenum* and *Trichophyton rubrum* obtained from S.M.S.lab. Jaipur (Rajasthan) were cultured on Sabouraud Dextrose Broth (SDB) at 37°C for 48 hr.

Cultures of Test Microbes

Stock cultures were maintained at 4°C on slops of nutrient agar. Active cultures for experiments were prepared by transferring an inoculating loop of cultures from the stock cultures to test tubes of SD Broth, which were incubated without agitation for 24 hr at 25°C. The Agar-well diffusion method [33] was used to screen for the antifungal activity.

Antifungal Assay

For antifungal assay Agar- well diffusion method was adopted, because of its reproductivity and precision. The plates were prepared by pouring 20 ml of molten media into sterile petri plates. The plates were allowed to solidify for 8 min. After that, 60 µl suspension was spread uniformly with the help of a sterile glass spreader and dried for 5 min. The wells (6 mm diameter) were punched in the plates using a sterile stainless steel borer. The test extract and control (ketoconazole) was loaded in a 6 mm well and the test sample was allowed to diffuse for 30 min. The plates were kept for incubation at 37°C for 36 hr. At the end of incubation, inhibition zones formed around the well were measured with a transparent scale in millimeters. The experiments were performed in triplicate and the mean value of the diameter of inhibition zones with ± standard deviation was calculated [34],[35].

Table-1 : Antifungal Activity of The Heartwood of Tectona Grandis Linn.

Plant Species: Tectona grandis (Heartwood)					
Test Microbes	**Extract/ Fractions**	**EtOH**	**Pet. Ether**	**C_6H_6**	**EtOAc**
	Dose (mg/disc)	**4**	**4**	**4**	**4**
P.crysogenum	**IZ**[+]	9.60±21	10.00±20	9.90±20	10.45±20
	AI*	0.32	0.33	0.33	0.34
C.albicans	**IZ**	8.98±22	9.60±22	10.00±20	9.06±21
	AI	0.40	0.43	0.45	0.41
T.rubrum	**IZ**	9.00±22	9.20±21	10.02±20	10.16±20
	AI	0.42	0.43	0.47	0.47
A.niger	**IZ**	9.04±20	8.90±20	9.68±21	10.06±20
	AI	0.33	0.33	0.36	0.37
A.flavus	**IZ**	-	-	8.76±22	9.60±22
	AI	-	-	0.32	0.36

[+]IZ = Inhibition zone (in mm) including the diameter of disc (6 mm);
*AI = Activity index = Inhibition zone of sample/Inhibition zone of standard;
Standard : Ketoconozole;
(-) = No activity.

RESULTS AND DISCUSSION

Fungi are found to occur everywhere in the environment and cause infection in plants as well as in animals. Species of *Candida* and *Aspergillus*, are the most common causing life-threatening infections like lung infection and ear infection. Hence, in the present study out of five, two species of *Candida* and two of *Aspergillus*, were used to screen the possible antifungal activity of plant extract. In spite of a large number of antifungal drugs in the market, but due to the development of resistance against existing antimicrobial drugs. There remains a need for drugs that are more effective and exhibit broad-spectrum efficacy.

The results of antifungal activities of the selected plant extracts have been presented in Table 1. The ethanolic and pet. ether fractions were not active against *A. flavus* but show wide potential against *T. rubrum.* The benzene and ethyl acetate fractions show stronge activity against *T. rubrum* while mild activity against *A. flavus* and *P.crysogenum.* Overall, the heartwood of *Tectona grandis Linn.,* show wide potential against *T.rubrum* and mild potential against *A.flavus.*

CONCLUSION

Currently, microbial infections like black fungus infection, have become a hazardous threat to the medical system. Therefore, screening of medicinal plants and herbs are required for discovering new antimicrobial agents with wide potential. In this study, antifungal screening of the heartwood of *Tectona grandis Linn.* was done by the agar well diffusion method. The result showed that the extract/fractions of the heartwood of *Tectona grandis Linn.* has effectiveness against mostly test fungi except for A. flavus. The extract and fractions of the heartwood has a wide antifungal potential against *C. albicans* and *P.rubrum*. Therefore, from this study it is concluded that an important antibiotic against *C. albicans* and *P.rubrum* might be yield from the heartwood *of Tectona grandis Linn.* Further investigations are necessary to evaluate the potential against other infectious diseases, especially for antiviral diseases.

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